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College of Agricultural Sciences
American Cocoa Research Institute

United States Department of Agriculture

The Penn State Program in the Molecular Biology of Cacao

The Pennsylvania State University
College of Agricultural Sciences
Department of Horticulture

Mark J. Guiltinan
Associate Professor of Plant
Molecular Biology
Director, American Cocoa Research Institute
Program in the Molecular Biology of Cacao
Department of Horticulture
Biotechnology Institute
306 Wartik Lab
University Park, PA16802
Telephone: 814-863-7957
Fax: 814-863-6139
E-mail: mjg9@psu.edu

Siela Maximova
Research Associate
American Cocoa Research Institute
Program in the Molecular Biology of Cacao
Department of Horticulture
114 Tyson
University Park, PA16802
Telephone: 814-863-7286
Fax: 814-863-6139
E-mail: snm104@psu.edu

Introduction

Theobroma cacao (cacao) is an ecologically beneficial crop in tropical agroecosystems, and can serve as a cash crop for farmers in developing countries. The growing demand for cocoa products coupled with increasing disease pressures has led to the concept that enhanced germplasm with disease tolerance and high yields could dramatically impact the lives of cacao farmers in a positive fashion. Utilization of such cacao trees will increase cacao-farming profitability, promoting expanded and sustainable production. Improved conditions for farmers and their countries will contribute to a more reliable supply of cocoa beans for chocolate manufacturers, a win-win situation for industry, the developing world and the ecosystem. Currently, a large consortium of researchers, government agencies including the USDA, The World Bank and USAID, and the chocolate manufacturing industry are developing a global plan for implementing research and development activities promoting sustainable cocoa production. "The goal of the research and technology transfer component of this initiative is to improve the well-being of smallholder farmers through development and transfer of technologies for sustainable tree crop systems that increase productivity, generate income, and protect the environment" (proceedings of the Sustainable tree crop development meeting, Arlington, VA, Oct. 19-21,1999). More information on the "The Sustainable Cacao Project" can be reviewed on the Internet at: http://www.acri-cocoa.org/ac_scp.htm. The research conducted at Penn State on methods for vegetative propagation of cacao and in developing new biotechnology tools for crop improvement and propagation are of major importance for development and fast multiplication of elite germplasm for the scientific community and the cacao farmers. The Penn State research teams participation in the international cacao initiative includes collaborations with different cacao research centers, an important component of this effort is focused on technology transfer and capacity building.

Background of Penn State Program

The American Cocoa Research Institute (ACRI), a consortium of chocolate manufacturing companies and The Pennsylvania State University established the Penn State Endowed Program in the Molecular Biology of Cacao in 1986. We operate at PSU with revenues from an endowment and with additional funds from ACRI, PSU, and USDA, with the broad mandate to improve cocoa production through research on *Theobroma cacao*, the chocolate tree.

Overall Program Goals:

- To stabilize and regionally diversify cocoa production

- To promote increased cocoa production proportional to demand
- To improve economic status of cacao farmers and producing countries
- To protect rainforest habitat and associated species through promotion of sustainable and profitable cacao production systems

Approach:
To enhance the development of cacao germplasm and its deployment through the combined approaches of biotechnology, applied horticulture and technology transfer. We have three major thrusts:

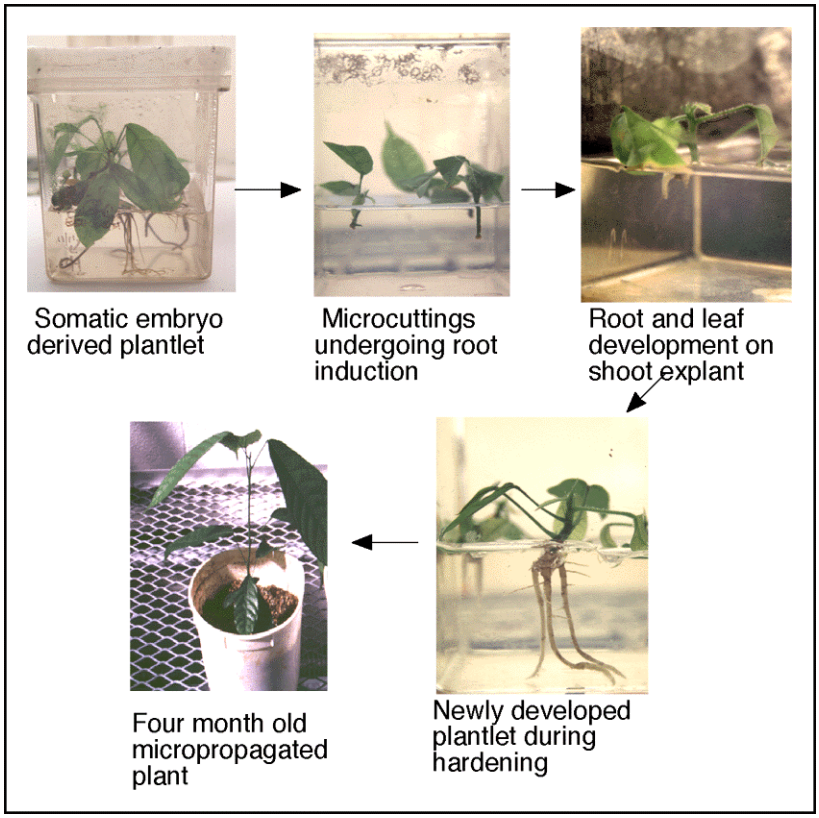
- Identification of Elite Cacao Germplasm
- Propagation Systems
- Technology Transfer

Propagation Systems Developed at PSU

We have developed four vegetative propagation methods that can be used in combination to achieve a large and rapid multiplication of cacao plants from single elite trees. These systems combine the advantages of *in vitro* tissue culture methods, with the simplicity and low costs associated with more traditional rooted cutting propagation. The systems include:

Somatic embryogenesis: A tissue culture based system starting from floral parts in which genetically identical embryos are formed. These embryos have the advantage of growing with the normal orthotropic-plagiotropic morphology of a seed grown cacao plant, unlike those made from cuttings or grafting of plagiotropic shoots. This growth form may have important advantageous over the plagiotropic plants in terms of normal tap root structure/function and lower pruning costs due to natural jourquette formation. We have begun field-testing of such plants in Saint Lucia and in Brazil.

Secondary embryogenesis: A process by which single somatic embryos can form multiple secondary embryos, each identical to the first. This was achieved by re-culturing explants from primary somatic embryos on induction media and secondary embryos are formed (Maximova et al., 2000). This procedure has an import scale up potential and provides a continuous source of embryos without the need to return to floral explants. It was estimated (using Scavina 6 genotype) that it is theoretically possible to produce over 4,000 secondary embryo derived plants from a single flower in approximately one year



Micropropagation: As somatic embryos are converted into plants, the small plantlets, still in tissue culture can be cut into portions each of which can form trees with normal growth morphology. This is a low cost and rapid scale up step.

Macropropagation: In the field, perhaps even in farmers hands, small plants can be induced to form orthotropic shoots, which can be excised and rooted, forming genetically identical plants. This is a very low cost method with a potential very high multiplication rate. We have developed a low cost misting system, which will make this method easily transferable to the field.

Integrated propagation system

Together, these methods can provide a means for rapid propagation of elite trees vegetatively from a limited starting material, and in a manner which produces trees with normal architecture. We believe that this system can have an important impact on farmers, providing inexpensive planting materials of high genetic potential. The tissue culture part of the system could be implemented in a University or government lab, and the macropropagation system can be distributed throughout the growing regions in small nurseries. Currently we have started the transfer of this technology to growing regions, and the development of such nursery facilities and demonstration plots is underway. Field testing of such plants is essential to completely validate the growth vigor and yield potential of plants produced by these methods. This will be done in side by side plots, with plants produced by other methods of propagation.

In November of 2000 a small field test was established in St Lucia. Additionally, some of the first somatic embryo-derived plants transferred to Saint Lucia are now

approaching 3 years of age, and appear to be quite healthy, have branched, flowered and have set fruit. Shown above is one such plant in St. Lucia at 1 1/2 years of age.

Technology Transfer Workshops, Scientific Exchange and field test establishment

CIRAD, FRANCE: The morphological and ultrastructural changes occurring over time during primary and secondary cocoa somatic embryogenesis were studied by Dr. Siela Maximova of Penn State in collaboration with Dr. Laurence Alemanno of CIRAD, using a combination of electron and light microscopy for the genotype Scavina 6 (Alemanno et al., 2000). This analysis showed that primary embryos arise predominantly from clusters of cells, which co-operatively form embryonic nodules perhaps as a result, from complex interactions of hundreds or thousands of cells. Interestingly, secondary embryos usually arise from the division of single epidermal cells, in a pathway reminiscent of zygotic embryogenesis. Thus, the two types of embryos originate through pathways that differ in the number and location of the cells contributing to embryo formation.

CATIE, COSTA RICA: A workshop on cocoa tissue culture was presented at the CATIE facilities in Turrialba, Costa Rica in July, 1999 which covered all aspects of our tissue culture methods. A training manual was developed and given to all attendees. Personnel from seven countries attended.

CEPLAC, BRAZIL; Workshop of Molecular Marker Technology and Cacao Tissue Culture: A series of two workshops were also presented at CEPLAC in Bahia, Brazil to a group of CEPLAC researchers. During these workshops, scientists and technicians were taught our tissue culture methods, and a large number of cultures were initiated. Additionally, training was provided on the use of an automated DNA sequencing apparatus for use in molecular marker analysis.

Centre for Cocoa Studies, M&M MARS, BRASIL: In collaboration with the MARS company we have established a large number of somatic embryo plants in the field in Bahia, Brazil. Additionally, Dr. Smilija Lambert of Mars Inc. in collaboration with researchers from Penn State (Lambert et al., 2000) completed work performed at the Almirante Centre for Cocoa Studies.

WEST AFRICA: Beginning in March of 2000, a Penn State research associate, living in Africa, has worked to establish the Penn State cocoa embryogenesis system in Ghana and Ivory Coast. At the end of the first year there are more than 800 somatic embryos produced in Ivory Coast and more that 400 embryos in Ghana. With support from Sustainable Tree Crop Program, USAID during year 2001, the technology will also be also transferred to scientist in Cameroon and Nigeria.

Visiting Scientists

We extend an open invitation to scientists interested in our technologies and willing to spend time with us at Penn State. To date, we have hosted scientists from Peru, Brazil, Cameroon, Vietnam, France, and England. Graduate students from Peru, and Ivory Coast

are currently working towards degrees in our program. We will host a scientist from Colombia in the summer of 2001.

Future Directions

Cacao Genomics

We plan to develop an international database of cacao gene sequences, which will provide a vast resource for breeders and researchers worldwide. To develop this database, the powerful new technologies of plant genomics will be used to identify many of the genes in cacao responsible for disease resistance and other characteristics. QTL mapping will greatly speed up plant breeding. Gene discovery will lead to new knowledge and tools to be used in improvement of cacao.

Virtual Cacao Research Network

One of the difficulties in cacao research is the vast distances and language barriers between producing countries and other research sites, which limits greatly the interactions between scientists that can greatly enhance research progress. We are planning a system of electronic communications that will enable researchers from various countries to come together for frequent discussions and presentations about the work and plans at various cacao research programs. A series of lectures and workshops will be presented via this system, and will be archived for viewing at later times.

Publications

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